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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/510,652	10/28/2004	Steven K. Libutti	230809	4377
36339 7590 05/22/2008 NATIONAL INSTITUTE OF HEALTH C/O NEEDLE & ROSENBERG, P.C. SUITE 1000 999 PEACHTREE STREET ATLANTA, GA 30309			EXAMINER SINGH, ANOOP KUMAR	
			ART UNIT 1632	PAPER NUMBER
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

### Office Action Summary

**Application No.**

10/510,652

**Applicant(s)**

LIBUTTI ET AL.

**Examiner**

Anoop Singh

**Art Unit**

1632

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --  
**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 19 March 2008.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 26-50 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 26-50 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SE/US)  
Paper No(s)/Mail Date \_\_\_\_\_
- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date \_\_\_\_\_
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_

### **DETAILED ACTION**

Applicant's amendments to the claims filed March 19, 2008 have been received and entered. Claim 26 has been amended, while claims 1-25 have been cancelled. Claims 26-50 are pending.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 3/19/2008 has been entered.

### ***Election/Restrictions***

Applicant's election with traverse of the invention of claims 41-50 (group II) filed October 4, 2006 was acknowledged. The traversal was on the grounds that Examiner has not set forth convincing argument that the search and examination of all the groups necessarily represents an undue burden for the examiner. Applicant's argument for examining remaining claims drawn to a method of measuring the angiogenic activity of a test molecule by comparing the fluorescence vascular density were persuasive since both sets of group embrace methods of measuring angiogenic or anti angiogenic activity. Therefore, invention of claims 26-40 (group I) directed to a method of measuring angiogenic activity by comparing fluorescence vascular density assay was rejoined with elected inventions of group II for the examination purposes. Applicants have also elected polypeptide, synthetic molecule, fluorescein, XTT, serum and filter paper as species for claims readable on claims 26-50.

Claims 26-50 are currently under consideration.

***Priority***

It was noted that instant application is a 371 of PCT/US03/10932 filed on 04/09/2003 which claims benefit of 60/371,010 filed on 04/09/2002. However, upon review the disclosure of the prior-filed application, US provisional application 60/371,010, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. It is noted that 60/371,010 dated 4/9/2002 describes measuring angiogenic activity using fluorescence vascular density but does not show support for a method to determine angiogenic activity using XTT. Consequently, there is no written description in application for using XTT or any other metabolic agent to determine angiogenic activity using spectrophotometer. In case, if applicants have evidence to support otherwise, applicants are invited to indicate page and line number for the written support as recited in claims 41-50 of the instant application. Therefore, the effective filing date for instant claims 41-50 is 04/09/2003 as subject matter of instant claims was described in the 60/371,010.

***Declaration***

The declaration filed on March 19, 2008 under 37 CFR 1.132 is not sufficient to overcome the rejection of claims 26-50 upon declaration of Dr. Cuttitta, applied under 35 U.S.C. 103(a) in part because of new grounds of rejection necessitated by claim amendments. The declaration will be discussed in detail below as it applies to the rejection.

***Withdrawn- Claim Rejections - 35 USC § 103***

Claims 26-34, 36-40 rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Science, 1994, 264, 570-571 IDS) and Robert et al (Cancer Res.

1992: 52(4): 924-30) and Kimel et al (SPIE, 1996, 2628, 69-76, IDS) is withdrawn in view of amendments to the claims. It is noted that claimed amendments requires digitally quantifying the plurality of pixel fro the three dimensional image to determine the FVD. However, upon further consideration a new rejection is made in view of amendments to the claims.

Claims 26-40 rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Science, 1994, 264, 570-571 IDS) and Rizzo et al (Microvascular Res, 1995, 49, 49-63, IDS) is withdrawn in view of amendments to the claims. It is noted that claimed amendments requires digitally quantifying the plurality of pixel fro the three dimensional image to determine the FVD.

***Maintained- Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 41-50 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Methods in Molecular Biology, 129, 257-269, IDS), Kurz et al (Developmental Dynamics, 1995, 203, 174-186), Frasca et al (Oncogene, 2001: 20, 3845-3856) and Kinnman et al (Lab Invest. 2001; 81(12): 1709-16, IDS) for the reasons of record.

Applicants' arguments and declaration filed March 19, 2008 have been fully considered but they are not fully persuasive. Applicants argue that one of skill in the art would have not presumed that XTT could be substituted for BrdU for

detecting proliferation in the CAM. Applicants assert that there is a significant difference between BrdU and XTT in measuring proliferation of cells. Applicants also argue that results of XTT would not discriminate between the cell type. Applicants assert that there is no evidence in prior art that present results could detect significant effect of angiogenic and anti angiogenic effects in CAM.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). It is noted that applicants agree that BrdU, XTT and MTT were commonly used assay to measure proliferation of cells but asserts that significant differences exist in detection method. However, it is well established in case law that a reference must be considered not only for what it expressly teaches, but also for what it fairly suggests. *In re Burkel*, 201 USPQ 67 (CCPA 1979). Furthermore, in the determination of obviousness, the state of the art as well as the level of skill of those in the art is important factors to be considered. In the instant case, Examiner has provided the reference of Brooks et al (1999) that teaches a method to measure angiogenic and anti angiogenic activity of a test molecule by obtaining a 10 day old chick egg wherein it is candled to determine prominent blood vessel and then via a small window of exposed area a filter disc is placed followed by systemic administration of test molecule (see Figure 1 pages 261-264). It is noted that Brooks et al also suggest filter disc saturated with test agent which could be angiogenic stimulator could be placed on the CAM (see Figure 1 page 263). Brooks et al also indicate that only up to 100µl of single injection could be administered to the vessel (see page 264, paragraph 1). Brooks et al also teach quantitation of angiogenic or anti angiogenic activity by removing the filter disc and associated CAM tissue that is placed on Petri dish for quantitation of number of blood vessels (see page 265, paragraph 1). While Brooks et al described the potential of measuring angiogenic

and anti angiogenic activity using CAM assay. Brooks et al differed from the claimed invention by not teaching use of adding an agent to measure metabolic activity to quantitate number of viable cells in the test area. Kurz cure the deficiency of Brooks by teaching that CAM endothelial cell proliferation is regulated by endothelial cell density and proliferation of endothelial cells should be used for the evaluation of angiogenesis in CAM assay (see abstract). Although, Kurz exemplified a method that differed from the claimed invention by not teaching administering an agent XTT or any other metabolic agent for measuring the metabolic activity in the test area. However, uses of XTT, MTT, WST-1or BrdU for measuring the cell proliferation was known to one of ordinary skill in the art at the time the claimed invention was made and these assays were routinely used in alternative to each other as evidenced by the teaching of Frasca and Kinnman.

Applicants argue that that there are significant differences between BrdU and XTT in measuring proliferation of cells. Applicant's point that BrdU based assay can be used with a cell specific antibody to discriminate the cell type, while XTT is indiscriminate.

In response, it is noted that applicant's arguments are not commensurate with the scope of the claims. In the instant case, contrary to applicant's assertion Kurz et al teach VEGF induced pattern of DNA synthesis in endothelial cells at different days (see page 185, col. 1, para. 3). Specifically, Kurz et al show that BrdU can be incorporated into the newly synthesized DNA of replicating cells during the S-phase of the cell cycle by substituting for thymidine during DNA replication and using antibodies specific for BrdU can then be used to detect the incorporated chemical indicating cells that were actively replicating their DNA (see page 177, col.1, para. 3 and page 175, col. 2, last para.). Based on preceding discussion it is apparent that Kurz emphasized the importance of measuring proliferating cells in CAM to measure the angiogenic index. Although BrdU method taught by Kurz could also be used to further characterize the cell type and exact role of cell type in

angiogenesis using histology or double staining at, but this does not negate the fact that instant method of Kurz emphasized measuring proliferation in VEGF induced CAM to measure the extent of angiogenesis. Therefore, given that many methods to measure proliferation of cell such as XTT, MTT or BrdU were commercially available, it would have been obvious for one of ordinary skill in the art to for to use BrdU or any equivalent method to measure proliferation of cells such as method that use metabolic agent to determine cell viability/proliferation in order to determine the density of proliferating cells as an index of angiogenic activity in the method of Brooks with reasonable expectation of success.

Applicants argue that XTT method is indiscriminate and would not distinguish the cell population like BrdU. Applicants' also argue that Kinnamn and Frasca report measuring proliferation of cells in homogenous population of cell and not in heterogeneous tissue. In response, it is noted that applicants' arguments are not commensurate with the scope of the claims. In the instant case, none of the method claims require discriminating or indiscriminating cells incorporating BrdU, MTT or XTT. The claims are not limited to stain any specific cell population and embrace a method that would measure absorbance to the extent it could be extrapolated to angiogenesis. In this regard it was previously indicated by the examiner that measuring proliferation of endothelial cells indirectly measures the vessel density for quantitation of angiogenesis in CAM assay (see Kurz). Kurz et al teaches <sup>3</sup>H thymidine labeled endothelial cells are evenly distributed throughout the CAM capillary network and CAM expanded by an overall proliferation of endothelial cells prior to 11 days of incubation (see page 175, col. 1, para. 1). Thus, cited art provide overwhelming evidence that extensive proliferation of endothelial cells in capillary tube in CAM around ~8-10 post incubation, therefore, any method that measure number of cells in capillary network of CAM would extrapolate to extent of angiogenesis. Thus, contrary to applicant's argument a method that measures number of cells in the capillary network of CAM would provide extent of



angiogenesis and it would have been obvious for one of ordinary skill in the art to substitute BrdU with another equivalent method such as XTT or MTT assay that is based on the ability of metabolic active cells to reduce the tetrazolium salt XTT/MTT to orange colored compounds of formazan that is water soluble wherein the dye intensity can be read at a given wavelength with a spectrophotometer. There is no evidence on record that any other cell type that does not contribute in the process of angiogenesis is present in the capillary region of the CAM in significant number. In fact, art of record teaches extensive proliferation of endothelial cells are evenly distributed throughout the CAM capillary network and CAM expands by an overall proliferation of endothelial cells i, it would have only required routine experimentation to modify the method disclosed by Brooks and Kurz to include XTT or other assays to measure proliferation of cells in capillary network of CAM to measure angiogenic or anti angiogenic activity as required by instant invention.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

### ***New-Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 26-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Methods in Molecular Biology, 129, 257-269, IDS), Iruela-Arispe et al

(Circulation. 1999; 100:1423-1431 ), and Rizzo et al (Microvascular Res, 1995, 49, 49-63, IDS).

Brooks et al teach a method to measure angiogenic and anti angiogenic activity of a test molecule by obtaining a 10 day old chick egg wherein it is candled to determine prominent blood vessel and then via a small window of exposed area a filter disc is placed followed by systemic administration of test molecule (see Figure 1 pages 261-264). It is noted that Brooks et al also suggest filter disc saturated with test agent which could be angiogenic stimulator could be placed on the CAM (see Figure 1 page 263). Brooks et al also indicate that only up to 100µl of single injection could be administered to the vessel (see page 264, paragraph 1). Brooks et al also teach quantitation of angiogenic or anti angiogenic activity by removing the filter disc and associated CAM tissue that is placed on petri dish for quantitation of number of blood vessels (see page 265, paragraph 1). While Brooks et al described the potential of measuring angiogenic and anti angiogenic activity using CAM assay but differed from the claimed invention by not teaching use of directly injecting into vessel located in CAM a fluorescent labeled particle to quantify fluorescent vascular density.

Iruela-Arispe's teachings cure the deficiency of Brookes by teaching a method of measuring the effect of a test agent (TSP-1, fusion proteins, and peptides) on angiogenesis in a modified CAM assay . The method is based on obtaining a chicken egg and then the vertical growth of new capillary vessels into a substrate collagen gel pellet is placed on the CAM. The collagen gel is supplemented with an angiogenic factor such as FGF-2 (50 ng/gel) or VEGF (250 ng/gel) in the presence or absence of test proteins/peptides. The extent of the angiogenic response is measured by using FITC-dextran that is injected into the circulation of the CAM. Iruela-Arispe teaches that the degree of fluorescence intensity parallels variations in capillary density; the linearity of this correlation can be observed with a range of capillaries between 5 and 540. Additionally, analyses of angiogenesis is performed by

acquisition of images with a Sony, single-chip CCD camera and measurements of fluorescence intensity as positive pixels. Iruela-Arispe also teach comparing each data point with its own positive and negative controls present in the same CAM to determine percentage of inhibition, considering the positive control to be 100% (VEGF or FGF-2 alone) and the negative control (vehicle alone) (see page 1424, col. 1, last para.). Iruela-Arispe teaches same method steps to determine the effect of test agent to measure the angiogenic or anti angiogenic activity but differed from claimed invention by not disclosing capturing three dimensional pictures and digitally quantifying the plurality of pixel to obtain FVD.

Rizzo cure the deficiency of Iruela-Arispe by disclosing that the microcirculation within the chorioallantoic membrane (CAM) of the chick is particularly well suited for *in vivo* observation and has been used extensively as an assay to detect angiogenic activity. Rizzo et al teaches a method to quantitate the relative micro vascular permeability associated with tumorigenesis and normal angiogenesis by microinjecting a graded series of FITC-dextran into a vessel of CAM and then measuring the fluorescence by a confocal attachment to differentiate different capillary network (see page 50 bridging to page 51; materials and method). It is noted that Rizzo et al disclose that the tissue plane containing the CAM capillary networks is separated from that containing the first-order micro vessels by a distance of less than 5  $\mu\text{m}$ , optical dissection of the tissue planes is well within the theoretical z resolution ( $1 < \text{mm}$ ) of the confocal system. Following each microinjection, randomly selected fluorescent confocal images of the respective tissue planes is captured by the image-analysis software at 2, 5, 7, 10, and 15 min for play-back analysis (see page 51, para. 2 and 3). Additionally, Rozzo et al teach quantitation of changes in intensity by recording fluorescent images that are digitized for quantitative assessment of fluorescent intensity. The digitized images are composed of pixels of varying brightness depending on respective pixel light intensity (gray-scale levels ranged from 0 to 255) meeting the limitation of claims

37-40. Furthermore, mean values different capillaries are calculated for the comparison of the data (see page 51, para. 4 and 5). The results of Rizzo et al provide evidence that method to capture 3 dimensional image from the test region comprising plurality of pixels and digitally quantifying the plurality of pixel to obtain the fluorescence density was known in prior art to investigate the vasculature (see page 62, last two lines). Although, Rizzo did not study the effect of any agent on angiogenesis but provided adequate guidance to one of ordinary skill in the art to use suitable concentration of FITC dextran that could be used to determine perivascular interstitial intensity by recording fluorescent images that are digitized for quantitative assessment. Rizzo et al differed from the claimed invention by not teaching administering an angiogenic agent and measuring the fluorescence by confocal to measure the angiogenic or anti angiogenic activity.

Accordingly, in view of the teachings of Brooks, Iruela-Arispe and Rizzo, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or anti angiogenic activity in a CAM assay of Brooks by measuring fluorescent vascular density by directly injecting FITC-dextran into the circulation of the CAM as disclosed by Iruela-Arispe with a reasonable expectation of success particularly since it is disclosed that the degree of fluorescence intensity parallels variations in capillary density. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Iruela-Arispe had already disclosed that FITC-dextran could be microinjected to determine the vascular density in CAM to compare the angiogenic or anti angiogenic activity (supra). Although Iruela-Arispe did not capture 3 dimensional image to digitally quantify the pixels to obtain FVD but given the teaching of Rizzo one of ordinary skill would conclude that using any commercially available confocal LCM including one taught by Rizzo would have provided 3 dimensional pictures comprising plurality of pixel to digitally quantify the pixel to obtain FVD as disclosed by Rizzo. It would have obvious for an artisan

to administer FITC-dextran directly into the vessel as taught by Iruela-Arispe and obtain 3 dimensional image of cells comprising plurality of pixel to digitally quantify to obtain FVD as generally known in art and taught by Rizzo to determine fluorescence using the method of Brooks/ Iruela-Arispe to measure angiogenic or anti angiogenic activity as disclosed in the instant application.

One who would practiced the invention would have had reasonable expectation of success because Iruela-Arispe /Brooks had already taught a method to measure angiogenic or anti angiogenic agent activity in a CAM assay. Iruela-Arispe /Rizzo et al had already described use of FITC-dextran to measure the bio distribution in neo vasculature in CAM that could have been used as for quantitation to compare angiogenic or anti angiogenic activity. Thus, it would have only required routine experimentation to modify the method disclosed by Iruela-Arispe /Brooks and Rizzo to include capture the image with CLCM comprising plurality of pixel to digitally quantify the plurality of pixel from the 3 dimensional image to obtain FVD to measure angiogenic or anti angiogenic activity as required by instant invention.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 41-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Methods in Molecular Biology, 129, 257-269, IDS), Kurz et al (Developmental Dynamics, 1995, 203, 174-186), Yasukawa et al ( Invest Ophthalmology Vis Sci, 1999, 40: 2690-2696) and Woltering et al (US Patent 6,893,812, dated 5/17/2005, filed 5/25/2001, effective filing 5/30/2000).

Brooks et al teach a method to measure angiogenic and anti angiogenic activity of a test molecule by obtaining a 10 day old chick egg wherein it is candled to determine prominent blood vessel and then via a small window of exposed area a filter disc is placed followed by systemic administration of test molecule (see Figure

1pages 261-264). It is noted that Brooks et al also suggest filter disc saturated with test agent which could be angiogenic stimulator could be placed on the CAM (see Figure 1 page 263). Brooks et al also indicate that only up to 100 $\mu$ l of single injection could be administered to the vessel (see page 264, paragraph 1). Brooks et al also teach quantitation of angiogenic or anti angiogenic activity by removing the filter disc and associated CAM tissue that is placed on petri dish for quantitation of number of blood vessels (see page 265, paragraph 1). While Brooks et al described the potential of measuring angiogenic and anti angiogenic activity using CAM assay. Brooks et al differed from the claimed invention by not teaching use of adding an agent to measure metabolic activity to quantitate number of viable cells in the test area.

However, prior to instant invention was made, use of proliferation-based assays was routine in the art to quantitate angiogenic or anti angiogenic activity. Kurz et al cure the deficiency of Brooks by teaching a method to analyze the density and distribution of whole mount BrdU anti BrdU labeled endothelial cell in a CAM with computer assisted microscopy. It is noted that Kurz et al taught a method to obtain CAM at different days (see page 175, col. 2, para. 3) that were analyzed for the influence of VEGF in proliferation intensity. Kurz et al also described the nuclear incorporation (metabolic degradation) of BrdU is not as rapid in avian cells (see page 182, col. 1, para. 1, and Figure 5). It is emphasized that Kurz et al proposed that CAM endothelial proliferation is regulated by a factor such as endothelial cell density of pre capillary vessel and length density of pre-capillary vessel, which should be used for evaluation of angiogenesis in the CAM assay (see abstract).

Kurz differed from the claimed invention by not teaching administering an agent XTT and measuring the metabolic activity to measure the metabolic activity of cell in the test area.

However, the uses for XTT, MTT, WST-1 or BrdU for measuring the cell proliferation was known in the art at the time the claimed invention was made and these assays were routinely used in alternative to each other. Yasukawa et al supplements the teaching of Kurz by disclosing a method that uses a tetrazolium - based colorimetric system, to measure human umbilical vein endothelial cell (HUVEC) growth in association with anti-angiogenic compounds (abstract). The method describes addition of an agent XTT after the addition of test molecule and described the method to measure and compare the metabolic activity at a specific wavelength (450nm) (see page 2691, col.2, para. 2), col. 1, para. 3, and Fig. 3). In addition, Woltering et al provided guidance with respect to methods to evaluate neovessel proliferation/promotion by either subjective scoring or by measuring cellular viability using any of various methods known in the art including colorimetric assay to measure metabolic activity of cells in a tissue fragment. Woltering et al embraced the potential of performing at the end of a specified time period on both the tissue fragment and on angiogenic sprouts (see col. 10, lines 30-39).

Accordingly, in view of the teachings of Brooks, Kurz, Yasukawa and Woltering, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or anti angiogenic activity in a CAM assay of Brooks by measuring metabolic active endothelial cell density in CAM tissue with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Kurz had already disclosed that proliferative pattern and the length density and extension should be used for the evaluation of angiogenesis in the CAM (see page 174, abstract, last 5 lines) and particularly since both Kurz and Brooks et al sought to quantitate angiogenic or anti angiogenic activity. Although Brooks or Kurz et al did not use XTT, Kurz generally embraced potential of measuring proliferation assay to better measure and compare angiogenesis. In

addition, Kurz provided motivation of measuring proliferation of cells to measure the vessel density for quantitation of angiogenesis in CAM assay (supra). Therefore, given that many methods to measure viability and proliferation of cell including XTT, Brdu were available during morphogenesis to compare the angiogenic activity of test molecule as per the teachings of Yasukawa and Woltering it would have obvious for an artisan to use XTT or any other assay to determine cell metabolic activity to measure angiogenic or anti angiogenic activity as disclosed in the instant application. It is noted that the one of ordinary skill in the art would have further motivated to optimize the treatment routes, regimen and would have optimize the steps of administering test molecule and agent to measure metabolic activity in different vessel depending upon total volume of test agent required for the angiogenic or anti angiogenic response as per the teachings of Brooks (see MPEP 2144.04). One who would practiced the invention would have had reasonable expectation of success because Brooks had already a method to measure angiogenic or anti angiogenic agent activity in a CAM assay. Kurz and Woltering had already described use of proliferation/viability assay to determine number of endothelial cell pattern during morphogenesis that could have been used as for quantitation to compare angiogenic or anti angiogenic activity. Thus, it would have only required routine experimentation to modify the method disclosed by Brooks and Kurz to include XTT or any other metabolic active agent to measure angiogenic or anti angiogenic activity as required by instant invention.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

### ***Response to Arguments***

It is noted that applicants' have argued and presented declaration of Dr. Cuttitta in support of novelty of the quantitative angiogenic assay by presenting evidence that it would have not been obvious for one of ordinary skill in the art



would to combine the cited references of Brooks, Robert, Kinel Rizzo and others to arrive to instant invention. Applicants' arguments and declaration have been fully considered and will be discussed to the extent it reads on references used in the instant rejection. It is noted that applicants' argument with respected to references of Roberts, Kinel and others are moot as these references have not been used for the rejection of pending claims. Applicant's arguments and declaration filed March 19, 2008 have been fully considered and they are not persuasive. Instant response is limited to the extent arguments are limited to applied reference.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e. introducing anti angiogenic reagent intravenously) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Additionally, contrary to applicants' argument Brooks et al contemplate introducing test agent via topical or systemic administration in the CAM (supra).

The declaration by Dr. Cuttitta's argues that prior to publication of Libutti et al assay, no one had successfully attempted to use direct injection of fluorescent molecule to quantitate angiogenesis even though such direct injection was already being used in other context.

In response, it is noted that contrary to applicant's argument, Iruela-Arispe taught a method of directly injecting FITC-dextran into the circulation of the CAM to determine the degree of fluorescence intensity parallels variations in capillary density. Iruela-Arispe disclosed the linearity of this correlation could be observed with a range of capillaries. Additionally, it is also disclosed that analyses of angiogenesis is performed by acquisition of images with a Sony, single-chip CCD camera and measurements of fluorescence intensity as positive pixels. Thus, it is

reasonable to conclude that method of introducing fluorescent molecule directly into vessel to quantitate angiogenic vessel was known in prior art.

Applicants' argument of optimization of different parameters including determining vein, the injection site, identifying size of needle, introducing anti angiogenic agent to minimize leakage, best size of FITC-dextrin dosage, drug toxicity level of the CAM, best time to harvest the CAM disk harvest after PITC intravenous injection to maximize fluorescent signal to validate assay reliability is not persuasive because these arguments are not commensurate with the scope of the claims. It is noted that none of the features argued by applicants are recited in the rejected claims. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

The declaration of Dr. Cuttitta asserts the commercial successful method based on recognition of superior results and use of instant method in wet lab course offered by FAES, NIH, Bethesda as evidence of commercial success (See page 4, section 9 and 10 of the declaration). Applicants argue that this commercial success is directly related to superior results of the assay over the other methods previously used.

Applicants' arguments and declaration is acknowledged but are not fully persuasive. In the instant case, applicants have asserted that "use of instant method in wet lab course offered by FAES, NIH, is summarized". However, recitation of success of the claimed method being taught at one institution is not a hard evidence of commercial success of claimed method using instant method for high throughput screening. In the instant case, it is unclear which method that uses specific concentration of specific fluorescent molecule covered by the commercial success particularly since pending claims are broad and embraces several variables as disclosed in this application and declaration (including injection site, needle size, type and size of fluorescent molecule). The assertion does not provide any hard

evidence or nexus between collaborative partners, licensees to the claimed method using any specific method that uses specific method steps commensurate with full scope of the claims. MPEP 716.03 states “[A]n applicant who is asserting commercial success to support its contention of nonobviousness bears the burden of proof of establishing a nexus between the claimed invention and evidence of commercial success”. In addition “Objective evidence of nonobviousness including commercial success must be commensurate in scope with the claims. *In re Tiffin*, 448 F.2d 791, 171 USPQ 294 (CCPA 1971)”. It is noted that applicants have failed to establish any nexus between licensing or collaboration with other entities and claimed method commensurate in the scope of the claims to suggest commercial success of the any of the claimed methods.

### ***Withdrawn Double Patenting***

Claims 26-50 provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-2, 7-20-22 and 27-35 of copending Application No. 11/014472 is withdrawn in view of amendments to the independent claims.

### ***Double Patenting***

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In*

re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 26-50 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 4-34 of copending Application No. 11/014472.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both sets of claims are directed to a method of measuring the angiogenic or anti angiogenic activity of a test molecule in a CAM assay by administering a fluorescent-labeled particle and measuring the FVD value or by using an agent that has metabolic activity and measure spectrophotometer reading to determine angiogenic activity. Since the specification and claims of the '472, application contemplated same test molecule and fluorescent-labeled particle or by using XTT and embraced same method steps in CAM assay as one disclosed in instant application. Thus, the claims of application no 11/014472 differs only with respect to a narrower scope of test molecule that is obtained from an animal that could be used in the method as claimed in instant application.

This is a provisional double patenting rejection since the conflicting claims have not in fact been patented.

It is noted that applicants have indicated that instant rejection would be addressed once claims are found allowable in either application.

***Conclusion***

No Claims allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Coagswell et al SPIE Vol. 2184 Three-Dimensional Microscopy , 1994, 49-54) teaches method of using 3 dimensional LSM to digitally quantify the fluorescence form a image.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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